**Case 13: X-linked Lymphoproliferative Disease**

**Summary:** Immune response to viruses is to kill the infected host cells by cytotoxic T cells and NK cells and to reduce number of extracellular virus particles using Abs. Both the innate and adaptive immune responses control viral infections. NK cells (innate) are large granular lymphocytes that don’t express Ag specific receptors. Instead carry receptors that recognize virus infected cells and release cytoxic proteins that induce apoptosis and death of the infected host cell (perforin and grnzyme, also released by Tc cells). Response by NK cells doesn’t require any previous immunological experience with the virus and is important especially when the person encounters the virus for the first time. Virus specific Tc cells (adaptive) are generated during the primary immune response to the virus and specifically kill infected cells by release of cytotoxic granules. Naïve virus-specific CD8 T cells are activated to effectors through engagement of TCRs with a complex of virus-derived peptide and MHC1 molecules on the surface of APCs (DCs). Also need costimulation by the DC and costimulatory help from activated CD4 T cells. Memory Tc cells are also produced during the primary immune response so that upon reexposure to the same virus (either by reinfection from the environment or reactivation of a latent infection) the memory Tc cells can rapidly recognize and kill the infected host cells that display the viral Ags. Virus specific primary and secondary Ab responses are also important especially for viruses causing significant viremia.

EBV infects epithelial cells and B cells. Expansion is usually well controlled by Tc cells and NK cells. Primary infection triggers activation and division of B cells that are infected by the virus. The infected B cells express viral Ags that are targets for specific cytotoxic responses by the NK cells and Tc cells that keep proliferation of infected B cells under control. In most people, EBV infection remains asymptomatic or results in infectious mononucleosis which evenually subsides in 6-10 weeks. After resolution of the acute infection, the virus remains latent in B cells, salivary glands, and epithelial cells of the nose and throat and can be shed in saliva. Reactivation can occur later in life and is usually brought under control quickly by EBV specific memory Tc cells (cellular immune surveillance). Primary and acquired deficiencies in T cell function are associated with marked susceptibilty to lethal EBV infection.

In normal people, EBV infected B cells are targets for killing by NK cells and virus-specific effector Tc cells. Fate of potential target cells is determined by the balance of activating and inhibitory signals that are delivered to NK cells via NK surface receptors. Inhibitory receptors interact with MHC1 molecules and prevent NK cells from attacking normal uninfected cells. NK CD244 receptors are members of the SLAM (signaling lymphocytic activation molecule) family, which can act as an activating or inhibitory receptor depending on which intracellular signaling protein it is associated with. The cytoplasmic portion of SLAM receptors have tyrosine-containing motifs that provide potential docking sites for the intracellular adaptors SAP (SLAM associated protein, encoded by SH2D1A gene) or by an alternative adaptor protein EAT-2.

SAP2 is composed of a single SH2 domain and is found in all T cells, germinal center B cells, and NK cells. Also expressed in NKT cells (subpopulation of lymphocytes with features of both NK and T cells, express invarient TCRs recognizing glycolipids). SAP is unusual in that its single SH2 domain can both bind to the tails of activated SLAM receptors and recruit they cytoplasmic Src kinase Fyn to the receptor complex by interacting with the SH3 domain of Fyn.

B cells that are infected with EBV increase expression of SLAM family member CD48 on their surface, which interacts with CD244 on NK cells to provide an activating signal that enables the NK cells to kill the infected B cells. Signaling via CD244 is critical in driving NK killing of EBV infected target B cells. CD244 contains several tyrosines in its cytoplasmic tail, which become phosphorylated when the receptor is activated and serve as docking sites for cytosolic proteins containing SH2 domains, including SAP. SAP binds to these cytoplasmic tyrosines on CD244 and recruits the Src-family tyrosine kinase Fyn to the receptor complex to allow propagation of the activation signal.

SLAM family is also implicated in the function of Tc cells, with receptors such as SLAM/CD150 and CD244 on T cells interacting with SLAM family molecules on Ag presenting DCs, B cells, and monocytes. With the exception of the CD48/CD244 pair, SLAM family molecules are homotypic (interaction of identical molecules between 2 cells).

Deficiency in intracelular signaling in NK cells and T cells impacts the immune system’s ability to control the EBV infection. In very rare instances, acute EBV infection in boys isn’t contained and results in a failure to eliminate the virus. Accompanied by massive overproliferation of lymphocytes (lymphoproliferation), overproduction of cytokines, destruction of liver and bone marrow, B cell lymphoma, and/or dysgammaglobulinemia (selective deficiencies of 1 or more, but not all, classes of Igs). Most cases result in death.

Most cases of X linked lymphoproliferative syndrome are due to SH2D1A gene defects, which encode for the signaling protein SLAM-associated protein (SAP). Patients with this defect show uncontrolled T cell activation, espeically in response to infection, but a reduced ability to kill EBV-infected B cells. Boys with EBV induced fulminant IM and SAP mutation will have XLP. The IM that develops is usually lethal (57%). Of those who survived, half developed lymphomas and the other half became agammaglobulinemic as result of B cell destruction (bone marrow may be destroyed leading to fatal aplastic anemia).

In the absence of SAP, active Fyn isn’t recruited to CD244 and alternative pathways are activated that result in inhibition of killing by NK cells (phosphatase SHP-1 binds to the to the rec and interrupts the activating signal). Numbers of NKT cells are severly diminsted in XLP patients, possibly because the activation of kinase Fyn is essential for their development. Also, the Tc response is exaggerated which suggests that signaling via SAP noramlly downregulates this response. In absence of SAP, SLAM family receptor signaling is dysregulated leading to unchecked proliferation of Tc cells, but impaired cytotoxic function.

Overproduction of cytokines as result of uncontrolled T cell proliferation seems to be important in causing life threatening tissue damage in XLP patients. After infection with EBV, XLP patients suffer destruction of the liver that is probably due to uncontrolled cytokine-mediated injury. Plasma levels of T cell derived cytokines such as IFNg, IL2, and TNFa are elevated (much lower or undectable levels in normal patients). The uncontrolled lymphocyte proliferation and cytokine secretion leads to syndrome of severe inflammation of the liver (hepatitis), destruction of bone marrow cells, and systemic shock. TNFa (from T cells) and IL1 (from monocytes) result in increased vascular permeability and loss of intravascular volume (analogous to toxic shock syndrome).

Tissue injury (especially in the liver) can also result from T cell derived cytokines promoting the expression of Fas on hepatocytes. FasL is expressed on the surface of activated T cells and can induce hepatocyte apoptosis. IFNg from T cells can also trigger the activation of monocytes/macrophages, which engaged in indiscriminate phagocytosis of surrounding cells. Histology often shows hemophagocytosis (macrophages engulf entire RBCs and other are laden with cellular debris). Hemophagocytosis is further enhanced because polyclonal B cell activation induced by EBV infection produced complement fixing Abs that interact with RBC Ags.

Although more than half of XLP patients are very suseptible to EBV, 1/3 develop dysgammaglobulinemia without an episode of severe mononucleosis. Experiments have shown that SAP is essential for the functioning of both T and B cell responses to soluble T dependent Ags, and needed for IgM and IgG responses to viruses, protein Ags, and haptens. Also needed for Ig class switching and germinal center formation. When SAP is absent both Th and B cells are functionally defective which may explain the progressive dysgammaglobulinemia in a subset of patients with XLP without involvement of EBV. SAP has a critcial role in development and function of follicular Th cells (subpopulation of T cells that secrete IL21 and govern recrutiment of Ag specific B cells to germinal centers to allow maturation of Ab responses). This SAP dependent deficiency of follicular Th cells accounts for dysgammaglobulimeia and imparied Ab responses observed in XLP patients due to SAP mutations. Impaired responses can precede the clinical manifestations.

XLP patients are also predisposed to B cell lymphoma. Most occurs because of B cell transofmration by EBV (classic t(8;14) chromosomal translocation of EBV induced Burkitt’s lymphoma). In some cases there is no direct evidence for EBV role in transformation. In these cases, altered or absent SAP function may interfere with tumor surveillance by interfering with the function of tumor specific NK cells. In some families, males with the idential SH2D1A mutation may be present with fulminant IM, dysgammaglobulinemia, or B cell lymphoma.

Minority of XLP patients carry a mutation for BIRC4 gene which encodes the X linked inhibitor of apoptosis protein (XIAP), whose function is to inhibit caspace-mediated apoptosis. Increased susceptibility to apoptotic stimuli.

XLP-1=SAP defect, XLP-2=XIAP defect

Autosomal recessive is more rare and can occur in females. Mutations in the IL2 inducible tyrosine kinase (ITK), which is activated in T cells in response to engagement of the Ag receptors. More commonly XLP symptoms are seen in girls due to defects in cytolytic machinery that result in hemophagocytic lymphohistiocytosis.

Diagnosis of XLP can be made using family history and classical clinical presentation (fulminant IM). Often there is no clear family history and the severe lymphoproliferation can’t easily be distinguished from other forms of malignant lymphohistiocytosis with hemophagocytosis. Molecular diagnosis using PCR analysis of the 4 exons encoding SAP. Flow cytometry and Western blotting can be used to identify patients where the SH2D1A mutation result in lack of SAP protein expression.

**Case:** 5 year old boy with persistent unexplained fever and abdominal pain, mildly enlarged lymph nodes on neck, decreased energy, past medical history included problems with persistent otitis media and several epidosdes of bacterial pneumonia between ages of 2 and 3, decreased blood IgG and normal IgA and IgM, family history included a maternal uncle who died of aplastic anemia following an acute febrile episode and maternal grandfather died with history of recurrent lympomas, height and weight in 25th centile for age, few scattered petechaie (skin hemorrhages) noted on legs and feet, supraclavicular, axillary, or inguinal nodes weren’t enlarged, tonsils were moderately enlarged but not red or inflammed, abdomen moderately distended and tender in RU quadrant, liver was enlarged and edge was palpable, mild anemia (decreases hematocrit), decreased platelete count, very high proportion of atypical lymphocytes, elevated ALT and AST (alanine and aspartate aminotransferases, liver damage), titer of IgM Ab against EBV viral capsid Ag was positive, anti-ACV IgG was lower, no Ab against EBNA and EA (consistent with acute EBV infection), EBV viremia detected by PCR. Chest X ray showed enlarged lymph nodes in mediastinum, ultrasound showed significant amount of free fluid in abdominal cavity (ascites) and enlarged liver, abdominal CT showed enlarged lymph nodes in retroperitoneum, fever persisted and liver dysfunction and ascites worsened, shock symptoms resembling sepsis with hypotension, poor circulation, and multi organ failure, died 10 days after hospitalization, infiltration of liver, spleen, and lymph nodes by mixed population of mononuclear cells including small lymphocytes, plasma cells, and lymphoblasts, infiltrates in liver associated with necrosis, bone marrow showed decreased number of erythroid, megakaryocytic, and myeloid cells along with increased numbers of histiocytic cells, lymphocytes, and plasma cells, none of the 4 exons encoding SAP could be amplified by PCR, no expression of SH2D1A mRNA as detected by northern blot

**Treatment:** antiviral ganciclovir and IV immune globulin to try to control EBV infection, can prevent by destruction of patient’s bone marrow by radiation of chemotherapy (busulfan and cytoxan) followed by administration of HLA-matched marrow from a normal sibling or unrelated donor to replace all lymphoid precursors with normal SAP-expressing cells of donor origin

**Explain that NK cells can kill targets via 1) an ADCC type mechanism and 2) after an inability to detect MHC class I after binding an activating ligand on the targets.**

**Describe how cytotoxic T cells kill cells after the TCR interacts with particular MHC class I molecules presenting a specific antigenic peptide.**

**Explain that NK cells kill target cells after binding of their activating receptors (e.g. NKR-P1 or FcR) to activating ligands on target cells, but this tendency to kill can be negated by signal coming from NK inhibitory receptors.**

**Describe that an NK cell is inhibited from killing a cell if the NK cell’s inhibitory receptors are engaged (typically by binding MHC class I molecules on the target cell).**

**Explain that, in X-linked lymphoproliferative disease, there is a mutation affecting a signaling pathway molecule (SAP) that normally mediates the activation of NK or CTL killing activities. The loss of function in this signaling molecule leads to ineffective killing of EBV-infected B. cell targets by both NK cells and CTLs.**

**Explain that persons who lack a functional SAP usually die of a fulminant EBV infection, agammaglobulinemia (following loss of B cells due to EBV), or B cell lymphomas (following transformation by EBV).**

**Case 14: Hemophagocytic lymphohistiocytosis (HLH)**

**Summary:** In cytotoxic killing by Tc cells and NK cells, perforin forms multimeric pores in the cell membranes that enable the delivery of other cytotoxic proteins from the killer cell into the target. Cytotic proteins are preformed and stored in endosomal lytic granules. Once activated, cytotoxic lymphocytes reorient the microtubule-organizing center of the cell toward the point of contact with the target to guid the lytic granules toward the contact point. The lytic granules dock at the membrane, perforin polymerizes and inserts into the target cell membrane to form the pores that connect the killer and target cells. Granzyme B and grnulysin are release through the pore into the target cell where they induce apoptosis.

Process of degranulation can be detected by the apperance of lysosomal membrane-associated glycoproteins (CD107a, CD107b, CD63) in the cell membrane. When the cell is at rest, they are located on the inner surface of the lytic granule membrane. On degranulation, they become exposed on the lymphocyte surface.

Other proteins that are needed for successful cell-mediated cytotoxicity include Rab27a GTPase that promotes docking of the mature cytotoxic granules to the cell membrane (defect causes Griscelli syndrome type 2, HLH and hypopigmentation), Munc13-4 protein that promotes priming of the cytolytic granules (defect causes FLH3, HLH), and syntaxins-11 and Munc18-2 which are both park of the docking complex to enable fusion of the secretory granules with the cell membrane (defects cause FLH4 and 5 respectively, HLH).

Both congenital and acquired forms of HLH are known. The congenital forms are known as familiar hemophagocytic lymphohistiocytosis (FHL). Acquire HLH may be secondary to infections, malignancies, or autoimmune diseases. Patients with juvenile arthritis or SLE can develop a similar disease called macrophage activation syndrome. HLH is aggressive and potentially life threatning.

FHL is a rare disorder. Typically appears in infancy or early childhood and both sexes are affected. Transmitted as autosomal recessive. Clinical presentation includes high and persistent fever, spleen and liver enlargement, neurological manifestations (seizures, confusion, coma, associated with pleocytosis in CSF, also known as lymphocytic meningitis), severe anemia and thrombocytopenia, abnormal liver function, and coagulopathy (decreased fibrinogen and increased levels of fibrin degradation products makes the blood less able to clot), increased levels of serum triglycerides, and increased levels of inflammatory markers (ferritin and C reactive protein).

Symptoms of fever, immune activation, and increased inflammation are precipitated especially by infections with herepesviruses (CMV, EPV, varicella-zoster virus). These epsidoes are known as the accelerated phase of the disease. They can lead to multiple organ failure and death and can occur multiple times in the patient’s life.

Manifestations are consequence of the defect in lymphocyte cytotoxicity that makes patients unable to kill virus-infected cells. Viral infections lead to the activation of CD8 T cells that undergo clonal expansion. Continuous activation of NK cells and CD8 T cells leads to their infiltration into the liver, spleen, bone marrow, and CNS and secrete high amounts of IFNg. IFNg is a potent activator of macrophages which are induced to secrete pro-inflammatory cytokines such as IL6 and TNFa leading to tissue damage (cytokine storm). Activated macrophages lead to hemophagocytosis (phagocytic destruction of RBCs). Increased levels of soluble IL2R is also characteristic of HLH during active phases of the disease and is a marker for T cell activation.

At least 5 varients of FHL are known. FHL2 is due to the inability to produce a functional perforin. Deficiency in perforin doesn’t affect the formation of the cytolytic granules but impairs the release of cytotoxins and their entry into the target cell.

Other genetic defects include Chediak-Higashi syndrome (defect in LYST protein needed for protein sorting, HLH, hypopigmentation, giant lysosomes and peripheral neuropathy) and Hermansky Pudlak syndrome type 2 (defect in AP3B1 protein needed for protein sorting, HLH and hypopigmentation, neutropenia and tendency to bleeding due to functionally abnormal platelets). All disorders involving defects in cytotoxic machinery are characterized by increased susceptibility to viral diseases and overwhelming inflammatory response with increased production of IFNg.

Hypopigmentation in Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2, and Chediak-Higashi syndrome are due to the role of the affected proteins in melanogenesis (melanin production).

Diagnosis of HLH is based on a combination of clinical and laboratory features. In FHL there is a genetically determined defet in lymphocyte-mediated cytotoxicity. NK cell mediated cytotoxicity is usually assessed by culturing the patient’s peripheral blood mononuclar cells with Cr-labeled K562 target cells. If the patient’s NK cellshave intact cytotoxic activity, the Cr is released into the supernatant. Any defect in activation, docking, or priming of lytic granules can be detected by flow cytometric analysis for the appearance of CD107a on the surface of patient’s lymphocytes activated in vitro. This test would give a normal result in patients with FLH2 because the defect is in performin. Flow cytometry may be used to dianose FLH2 by intracellular staining for perforin.

**Case:** 2 mo. Old boy, parents distantly related, presented with rhinorrhea and fever, difficulty feeding, enlargement of liver and spleen, marked lymphocytosis, thrombocytopenia, anemia, CSF showed mild pleocytosis (increased WBC count), increased liver enzymes, markedly high ferritin, positive C reactive protein, elevated fibrin degradation products (coagulopathy), treated with ampicillin and amikacin but consciousness rapidly deteriorating, MRI with T2 showed spotty high density lesions in the white matter of cerebrum, basal nuclei, and cerebellum, high serum levels of triglycerides, low fibrinogen, bone marrow aspiration showed hypocellularity with increased number of large granular lymphocytes and macrophages with hemophagocytic activity, flow cytometry show absence of perforin, absence of NK cell mediated cytotoxicity demonstrated using K562 target cell line, sequence analysis showed single NT deletion in exon 2 resulting in a frameshift and production of an N terminally truncated perforin protein that couldn’t be detected by flow cytometry

**Treatment:** aggressive immunosuppression to stop the ongoing immune activation (remain highly prone to other episodes of accelerated phase leading to a high mortality rate), chemotherapy (dexamethazone, etoposide, cyclosporin A via the HLH 94 protocol)only cure is through hematopoietic stem cell transplantation with conditioning regimen based on busulfan, cyclophosphamid, and etoposide and CsA for graft vs. host disease prophylaxis, administration of anti IFNg monoclonal Ab might be effective to achieve remission without risk of the side effects related to use of chemo, steroids, or other immunosuppressive drugs, nonfamilial secondary forms of HLH are treated based on elimination of trigger in addition to immune suppression

**Explain that the killing machinery of NK cells and cytotoxic T cells.**

**Describe the pathogenesis of hemophagocytic lymphohistiocytosis (HLH).**

**Explain why patients with hemophagocytic lymphohistiocytosis (HLH) develop hyperinflammatory response in spite of their immune deficiency.**

Still show hyperinflammatory responses because the Tc cells can still recognize virus-derived peptides in associated with MHC1 molecules on surfaces of infected cells, promoting their activation and proliferation. As part of this response, they secrete large amounts of IFNg which drives the production of proinflammatory cytokines by macrophages (IL6, TNFa).

**Explain why patients with hemophagocytic lymphohistiocytosis (HLH) develop enlarged liver and spleen.**

Typical of the accelerated phase of HLH is the result of marked expansion of CD8 T cells and accumulation of activated macrophages.

**Explain why patients with hemophagocytic lymphohistiocytosis (HLH) show hypocellularity in their bone marrow.**

Bone marrow is a target organ in HLH. Activated macrophages often engulf red cells, myeloid cells, lymphoid cells, and platelets, leading to bone marrow hypoplasia.

**Case 15: Chediak-Higashi Syndrome (CHS)**

**Summary:** Cytoplasmic vesicles are small membrane-enclosed sacs that participate in transport within the cell and with the exterior, storage of nutrients, digestion of cellular waste and foreign products, and processing of proteins. Serve as closed chambers foc ehimcal reactions that could damage the cell if they were to occur outside the vesicle. Correct trafficking of vesicles is as important as the function of the vesicle itself. Functions include exocytosis, endocytosis, phagocytosis, phagolysosomal killing via ROS superoxide and proteolytic enzmyes (lysozyme, defensins), antigen presentation, Tc and NK cytotoxic killing, pigmentation of the skin and eye, synapses between neurons, and platelet activation. Defect in the trafficking of vesicles can lead to disease affecting many different functions of the immune system and other organ systems.

Chediak-Higashi Syndrome is inherited as autosomal recessive. Recurrent bacterial infections, partial absence of pigmentation of skin, hair, and eyes (oculocutaneous albinism). Patients have a tendency to bleeding due to platelet dysfuction (mild to moderate). If patients survive into adolescence or early adulthood most develop progressive neurological defects such as cerebellar ataxis, CNS atrophy, seizures, peripheral neuropathy, and cognitive defects. Most patients undergo an accelerated phase of uncontrolled lymphocyte proliferation and lymphohistiocytic infiltration characterized by fever, lymphadenopathy, hepatosplenomegaly and pancytopenia (reduction of platelets, RBCs, and WBCs) which is usually lethal.

Diagnosis made by examination of peripheral blood smear for distinctive giant cytoplasmic granules (vesicles in leukocytes and plateletes). All cell show the abnormal clustering of giant lysosome-like vesicles around the nucleus. Similar finding in the beige mouse strain (hypopigmentation of coat) which lead to the identification of the human gene responsible for CHS named CHS1 (also called LYST, lysosomal trafficking regulator). Can also diagnose by microscopic observation of a hair shaft. CHS patients show speckled clumps of pigment rather than normal homogenous distribution. Hair bulb of CHS patients under EM show melanocytes with enlarged melanosomes with variable amounts of pigment.

Protein is part of the BEACH family of proteins involved in vesicle formation and trafficking. Suspected that abnormal organellar protein trafficking may lead to aberrant fusion of vesicles and failure to transport lysosomes to the appropriate location in the cell (protein sorting). Most CHS1 mutations are nonsense or null that lead to absence of the protein, although milder phenotypes due to missense mutations have been described.

Main immunological defect is in innate immunity, impating the first line of defense to pyogenic infections of the skin and respiratory tract. Infections are frequent and usually severe, beginning shortly after birth. Microbes include staph aureus, strep pyogenes, and strep pneumoniae and fungi (candida, aspergillus). Neutrophil counts are mild to moderately decreased as result of destruction of neutrophils in the bone marrow and have decreased intracellular microbicidal activity. Affected nueotrphils and monocytes have low chemotactic and migratory capacity due partly to the fact that large fused granules impair the cells’ ability to move. Tc cells and NK cells have severely impaired cytotoxicity because of their inability to secrete granules containing the lytic proteins granzymes and perforin. B cells are less able to load peptide onto MHC2 molecules and have decreased Ag presentation capacity.

Pigmentation defect is due to inability of melanocytes to transfer pigment containing secretory granules to keratinocytes and other epithelial cells. Platelet dysfunction due to reduction in platele dense bodies (vesicles) that participate in sustaining platelet aggregation. Neurological disease due to problems of vesicular trafficking in neurons and glia.

**Case:** 4 yr old girl, recurrent ear infections and pneumonia, cellulitis and lymphadenitis (lymph node infection) due to s. aureus, history of easy brusing and mild nosebleeds, bothered by bright lights, fair hair and skin (unusual for indian descent), parents were second cousins from India, had 2 healthy siblings and no family members with history of recurrent infections, thin and small for age, mild fever and slightly fast RR, silvery sheen in her hair, irises of eyes were gray, perforation and pus in R tympanic membrane, moderate cervical lymphadenopathy, crackles in R lower lobe, mild hepatosplenomegaly, chest xray showed consildation in R lower lobe and pleural effusion (confirmed pneumonia), pleural culture grew s. aureus (started on IV nafcillin), normal WBC count but neutrophils were mildly decreased, normal neutrophil function, peripheral blood smear showed giant cytoplasmic granules in leukocytes (stained positive with myeloperoxidase), developed progressive neurological disease in adolescence characterized by weakness tremors, and ataxia which confined her to a wheel chair

**Treatment:** prophylatctic antibiotics and aggressive management of infections, bone marrow transplantations to correct immunological and hematological defects (prevents accelerated phase), manifestatations in nonhematopoetic organs (particularly the oculocutaneous albinism and progressive neurological disease) aren’t corrected by bone marrow transplant

**Explain that role of intracellular vesicles in Chediak-Higashi Syndrome (CHS).**

**Describe the pathogenesis of Chediak-Higashi Syndrome (CHS).**

**Explain why patients with Chediak-Higashi Syndrome (CHS) show nitro blue tetrazolium (NBT) test is normal.**

Nitro blue tetrazolium test measures the capacity of lysosomes in a phagocyte to produce superoxide and other oxygen free radicals. Performed by adding yellow NBT dye to phagocytes that are then stimulated with a cell activator (phorbol myristate acetate). Activation of the NADPH oxidase enzyme complex in the lysosome by PMA leads to the production of the reactive oxygen intermediates that modify NBT into formazon which has a deep blue color. CHS affects the normal formation and traffic of vesicles in the cell but doesn’t affect the function of NADPH oxidase so their neutrophils are able to reduce NBT to a blue color during the test.

**Explain why patients with Chediak-Higashi Syndrome (CHS) develop the accelerated phase of CHS.**

Accelerated phase consists of massive organ infiltration by lymphocytes. Due to impaired lymphocyte cytotoxicity, althought the precise mechanism is unclear. May typically occur after infection with EBV, immune system is unseuccessful in killing the virus-infected cells and in attempt to control the infection lymphocytes proliferate without restraint. Similar process observed in other diseases with impaired cytotoxicity (FHL2, mutation in perforin gene).

**Explain why patients with Chediak-Higashi Syndrome (CHS) develop the neurological disease after bone marrow transplantation.**

Bone marrow transplantation is only able to correct defects that are due to cells of hematopoietic origin. Oculocutaneous albinism and the neurological fects are not due to dysfunction of these cells, so these abnormalities can’t be corrected by bone marrow transplant.

**Case 16: Wiskott-Aldrich Syndrome (WAS)**

**Summary:** Many T cell functions require the directed reorganization of the cell’s actin cytoskeleton immediately underlying the plasma membrane. This is a dynamic structure that undergoes rapid reorganization by the depolymerization and repolymerization of actin filaments. Cytoskeleton is linked to cell surface receptors so that events occuring at the membrane can affect reorganization of the cytoskeleton. For example, engagemtn of TCR causes the T cell to express CD40L which binds to CD40 on the B cells. Cross linking of T cell antigen receptors and coreceptors by Ag:MHC complexes lead to their aggregation at one pole of the T cell, with an accompanying concentration of the actin cytoskeleton at that point. Binding of a Th cell to a B cell through binding to TCRs to Ag:MHC complxes on the B cell also lead to reorganization of the actin cytoskeleton locally in the zone of contact, which in turn causes a microtubule-dependent mechanism to focus the secretory apparatus of the T cell on the point of contact with the B cell. Allows release of cytokines from the T cell to be directed at the contact point (such as IL4). Similar reoganizations occur when cytotoxic T cells contact the target cells.

T cells (and other cells) move in a crawling fashion. Movement needed to get from thymus into blood vessels and into lymphoid tissue, which requires active participation of the actin cytokeleton. Cell division induced by activation of T cells by Ag or nonspecific mitogens also involves the actin cytoskeleton (cell is divided into 2 by action of the contractile ring formed by actin filaments and myosin).

T cells from patients with WAS are deficient in all the normal cellular abilities that involve the actin cytoskeleton and in particular seem unable to successfully interact with B cells and other target cells. Because the WAS protein is broadly expressed withing the hematopoetic system, other blood cell lineages are also affected.

X linked inheritance. Bloody diarrhea, eczema, thrombocytopenia with small platelets, immunodeficiency. Mutation in the WAS gene impacts production of the Wiskott-Aldrich syndrome protein (WASP) which has homology with actin-binding cytoskeleton proteins involved in the reorganization of the actin cytoskeleton on WBCs and platelets. WASP is only expressed in WBCs and megakaryocytes (platelete precursors) which explains the restriction of its effects to the immune system and blood clotting functions. In affected patients, T cells and platelets are defective in number and function. T cell movement, capacity for cell division, capping of Ag receptors, and reorientation of the cytoskeleton on engagement with other cells are all impaired. Function of monocytes, macrophages, and DCs are also affected with significant defects in directional motility and phagocytosis. Cytotoxic function of NK cells is also impaired. B cell abnormalities.

WASP structure: C terminal portion is the VCA domain that binds actin related protein complex (Arp 2/3) and this binding initiates the nucleation of action. Guides branching of actin filaments as they polymerize to make new filaments. WASP resides in the cytoplasm in an inactive form because the C term acidic domain is bound to the GTPase binding domain so that inactive WASP appears as a hairpin. When G protein Cdc42 (in GTP bound form) binds to GBD, WASP springs open into a linear activated form and the VCA domain can bind to the Arp 2/3 complex. Amino terminal WH1 domain of WASP binds to WASP-interacting protein and this binding stabilizes WASP. Missense mutations in the GBD result in constititively active form of the protein (associated with severe cellular abnormalities espcially in myeloid cells, leading to severe congenital neutropenia and increased risk of myelodysplasia, preleukemic bone marrow condition). Amino acid substitutions in N domain result in decreased levels of WASP (responsible for milder WAS called X linked thrombocytopenia, suffer from chronic thrombocytopenia but less prone to severe infections, autoimmunity or malignancies). Differentiate between chronic idopathic thrombocytopenia purpura and XLT by measuring mean platelet volume (WAS and XLT patients are the only condition typically associated with low MPV).

Abnormally small plateletes are a distinct and characteristic feature of WAS. Destruction of platelets by the spleen is greatly increased (not sure what the spleen recognized as abnormal). The platelets in the circulation become spontaeously activated and they extrude their grnules (this is why they appear small). Splenctomy may increased platelet number but it also increases the risk of sepsis (this is why it isn’t a recommended form of treatment). T cells are also morphologicall abnormal- lose their surface microvilli and look bald.

Patients have increased susceptibility to pyogenic bacterial infections and opportunistic infections (including severe chick pox, HSV, and molluscum contagiosum-eczematous skin). Increase susceptibility to viral infections is at least partly due to impaired cytotoxic function of CD8 cells and NK cells (can’t attach to target cells). Antibody formation, particullaryl against carbohydrate Ag, is also defective (shown by no response to anti-pneumococcal vaccine and no Ab against blood group antigens which are complex carbohydrates).

Antibody responses to carbohydrate Ags are mostly restricted to IgM and IgG classes. Natural Abs of IgM include hemagglutinins and Ab against common blood borne pathogens. Production of these is dependent on normal development of the marginal zone of the spleen (takes about 2 years to mature in normal patients). WAS patients have severe abnormalities of their spleen and lack a marginal zone. Explains why there is a lack of IgM Abs against carbohydrate moieties. Failure to produce IgG2 Abs against capsular polysaccharides and blood group Ag in WAS indicates that production of these involves T cells help. Confirmed by the fact that patients who were onverted to partial T cell chimeras with bone marrow transplant (B cells still of recipient origin) was sufficient to clear the eczema and enable them to make Ab against carb Ags.

WAS patients typically show progressive decline in T cell count, often associated with poor control of viral infections (espeically EBV) which can infect B cells. When this happens B cells undergo polyclonal expansion which may eventually become oligoclonal or monoclonal, leading to the development of B cell lymphoma.

High frequency of immune complex disease and allergy in WAS may relate to defective function of regulatory T cells (may account for high frequency of autoimmune manifestations, especially cytopenias).

**Case:** 20 mo old boy, history of recurrent middle ear and URT infections, eczema (infected with s. auereus), asthma, bloody diarrhea, autoimmune hemolytic anemia (become very pale and had dark urine, positive Coombs direct Ab test showed this RBCs were coated with IgG, treated with corticosteroids), normal at birth, previous episodes of pneumonia, wheezing between respiratory infections showed he had asthma, low hemoglobin, normal WBC count, low platelets (thrombocytopenia) and were very small, normal IgG, low IgM, increased IgA and IgE, blood type O but no anti-A or anti-B isohemagglutinins present, ab response to pneumoccus only present for 2/7 serotypes that were contained in the vaccine (no change when given booster), normal numbers of circulating B and T cells, normal distrubution of CD4 and CD8 cells, in vitro proliferative response of T cells to mitogen phytohemagglutinin was diminished and mitogenic effect of anti-CD3 Ab was decreased, flow cytometric and western blot showed lack of expression of WASP protein (premature stop codon), after bone marrow transplant had fever, rash, and elevated liver enzmyes (GVHD, treat with metylprednisone), positive family history (maternal uncle developed eczema, recurrent bronchitis, pneumonia, petechiae, thrombocytopenia, B cell lymphoma positive for EBV, died of sepsis by Gram negative enterobacillus)

**Treatment:** hematopoietic stem cell transplantation (require preior myeloablative treatment to allow robust engraftment of donor cells, cyclosporin A for prophylaxis against graft vs. host disease), regular administration of immunoglobulins, antibiotic prophylaxis (trimethoprim-sulfamethoxazole), measure to avoid severe trauma related bleeding

**Explain that the cortical actin cytoskeleton of a T cell becomes reorganized when a T lymphocyte interacts with other cells through surface receptors to become activated.**

Nonrandom X inactivation shown in heterozygous women. Signals that direct maturation of hematopoetic stem cells are transmitted by contact with bone marrow stromal cells. This interaction requires cytoskeletal reoritentation and will be impaired in cells containing active affected X chromosome, so stem cells with the normal active X chromosome have a survival advantage.

**Describe WAS as an X-linked immunodeficiency associated with mutations in WAS protein (WASP), that WASP is expressed in white cells and megakaryocytes, and its effects also are seen in platelets (which are derived from the latter).**

**Explain that an impairment of actin reorganization is seen in WAS-affected cells, and that most activities of T cells include cell interactions involving cell attachment, where actin is reorganized. For example, cell targeting by CD8 cytotoxic cells, as well as interaction of CD4 cells with B cells (CD40L on the T cells binding to CD40 on B cells, leading to reorganization – see image of talin in Fig. 22.2).**

**Explain that defects in both humoral and T-cell mediated immunity (especially viral infections where CD8 cells would be needed for control) are found in WAS.**

Could try to stimulate isotype class switching in B cells by giving an Ab against B cell surface protein CD40 along with the immunogen. Ligation of CD40 by CD40L on the T cell is a signal to the resting B cell that it should start dividing and undergo isotype switching. The Ab should act like CD40L and induce isotype switching in B cells.

**Case 24: Interferon- receptor deficiency**

**Summary:** Pathogens such as mycobacteria, Listeria, Leishmania, and Salmonella take up residence in macrophages and are protected from elimination by Ab or Tc cells. Eliminated only when their host macrophages are activated and produced increased amounts of NO, O radicals, and other microbicidal molecules. Macrophage activation in an adaptive immune response is directed by Th1 cells which produce IFNg. Macrophages take up micorbes by phagocytosis and produce IL12 which is necessary for the induction of IFNg synthesis by T cells and NK cells (also produce TNFa which acts autocrine to further stimulate itself and stimulate the T cell to express CD40L). Also favors the maturation of Th1 cells (which active macrophages) and suppresses maturation of Th2 cells (which secrete IL10, involved in deactivation of macrophages). Acitvated macrophage also increases expression of CD30 which interacts with CD40L on T cells to upregulate B7 expression and increasess expression of MHC2 molecules on the macrophage to allow further activation of resting CD4 T cells.

IFNg acts as a receptor on macrophage surface with 2 different types of polypepetide chains. IFNR1 and IFNR2 are each associated with a tyrosine kinase (JAK1 and 2). Dimer of IFNg binds to 2 molecules of IFNgR1 and cuases them to associated with 2 IFNgR2 chains which initiates a signaling pathway to result in changes in gene transcription.

AIDS epidemic has increased incidence of mycobacterial disease to to M. tuberculosis and others such as M. avium intracellular which are called atypical mycobacteria. Generally atypical mycobacteria don’t cause disease in normal patients except for swollen lymph nodes in which these mycobacteria survive. Genetic defect in IFNgR1 gene on chromosome 6 can also lead to susceptibility. Also can lead to progressive mycobacterial infection after immunization with BCG vaccine.

Other defects include defects in synthesis of p40 subunit shared by IL12 and IL23, or the IL12R beta chain (shows dependence of IFNg synthesis on IL12). Also mutations of STAT1 TF that interacts with IFNgR and mediates expression of IFNg responsive genes, and NEMO (IKKg) which regulates activation of NFkB and production of IL12.

IFNgR deficiency can be inherited as either autosomal recessive (IL12, IL12Rb1) or autosomal dominant and may be partial or complete. Dominant negative heterozygous STAT1 mutations cause MSMD, complete STAT1 deficiency inherited as autosomal recessive and causes MSMD associated with severe susceptibility to viral infections, NEMO deficiency is X linked disorder.

**Case:** 2 yr old female, from Maine, parents distantly related, well at birth, not eating well, diarrhea, weight loss, enlarged lymph nodes, CT showed mesentary and para aortic enlarged lymph nodes, slightly elevated monocytes, elevated IgG, IgA, IgM, biospy os nodes showed proliferation of histioncytes and neutrophils, acid fast stain for mycobacteria revealed numberous microorganisms and MAC was culture from the nodes and blood, developed iniltrates in lungs and progressive spleen enlargment, sepsis at age 6 and salmonella was cultured from blood, died from meningitis, MAC cultured from CNS, family history showed cousins who died of mycobacterial infections.

AIDS patients have issues clear mycobacterial (particularlly atypical) because the infection is normally contained by T cell action. Reduced CD4 Th1 cells prevents IFNg production and macrophages aren’t activated to clear the infection.

IFNg def patients would still show delayed type hypersensitive reaction with a PPD test. After the few specific tuberculin T cells bind Ag, they secrete chemikines that nonspecifically recruit macrophages and other inflammatory cells. Secretion of chemokines isn’t dependent on IFNg.

Wouldn’t see granuolma formation because they form where there is local persistence of Ag, Ag specific T cells and activated macrophages. IFNgR deficient patients couldn’t activate their macrophages so its not surprising that they didn’t form granulomas and that’s why mycobacteria was seen in their blood. Granuloma formation is preserved in autosomal recessive partial IFNgR1 def and IL12Rb1 def.

Wouldn’t have an issue with pyogenic bacteria such as pneumocci because it is controled by Ab and complement which is fine (even increased sue to increased IL6 production induced by mycobacterial infection). Viral infections such as varicella are terminated by Tc cells which are activated independent of IFNg. Salmonella are intracellular infections in macrophages (inaccessible to Ab and complement) that can only be detroyed by activation of macrophages by IFNg.

**Describe that IFN- is the dominant cytokine from Th1 cells that causes activation of macrophages.**

**Explain that macrophages secrete IL-12 upon ingestion of microbes, which subsequently induces IFN- production by Th1 and NK cells.**

**Explain that IFN-receptors on macrophages are composed of 2 polypeptide chains, and that ligand binding leads to macrophage activation via a JAK-STAT system, where tyrosine kinases (Jaks) are associated with each chain of the receptor.**

**Describe why lack of IFN-receptor leads to great susceptibility for mycobacteria infections, including BCG (the live vaccine strain for tuberculosis) and *Mycobacterium avium.***

**Explain that failure to produce either IL-12 or the IL-12 receptor is associated with susceptibility to BCG.**

**Describe that erroneous immunization of children displaying severe combined immunodeficiency disease (SCID) can result in disseminated BCG infection.**